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# A SIMPLE METHOD OF CULTIVATING BACILLI, PREFERRING CONDITIONS OF PARTIAL ANAEROBIOSIS (*B. ABORTUS*, BANG; *B. BIFIDUS*, TISSIER)

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Nowak's<sup>1</sup> idea, that in the growth of the *bacillus subtilis* in an enclosed vessel an appreciable amount of oxygen is used, thereby bringing about a condition of partial anaerobiosis which in turn favors the growth of certain organisms, is founded on solid reasoning. In following Nowak's line of thought, numerous methods have been adopted which have met with more or less success. One method that commands attention is the placing of nutrient agar-agar in the bottom of a mason jar, sterilizing and then slanting it. After streaking the surface with the *bacillus subtilis*, agar slants, previously inoculated with suspected (abortion) material, are placed in the jar, which is then tightly sealed. The jar with its contents is incubated at 37° C. for varying lengths of time.

The one objection to this expedient, however, is that the time required for initial growth varies from three to ten days and in the larger number of cases from seven to ten days. To obviate the necessity of such delay in obtaining the results sought the following method was conceived:

Employ an ordinary large test tube such as is generally used in making potato cultures. In this insert a tightly fitting piece of thin glass which should be about three-fourths the length of the test tube. Place plain agar-agar, as culture medium in the tube and insert a cotton plug. Sterilize the tube thus prepared. After this process slant the tube so that the agar lies high on one side of the central glass slide. Carefully observe the tube and note this, as exactness in this regard has a direct bearing on the success of the technic. The water of condensation will tend to collect at the bottom of the lower side thus minimizing the chance of surface spreading or contamination of the higher side.

1. *Ann. de l'Inst. Pasteur*, 1908, 22, p. 541.

Streak the lower side with the *bacillus subtilis*, taking care to cover the whole surface. Inoculate the higher side with some aborted material, amniotic fluid from an aborted calf, or the fluid from the interior of a cyst of the ovary. After inserting the cotton plug, push it down into the tube and place a tightly fitting rubber cork over it to make the tube air-tight. The tube thus prepared must be incubated at 37° C.

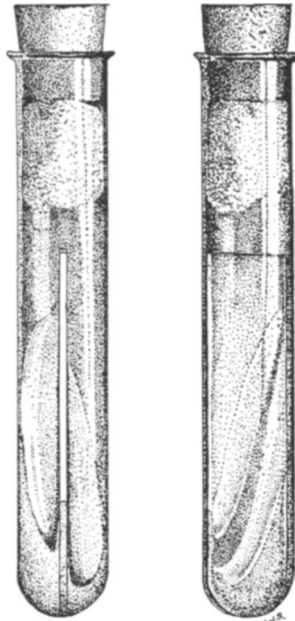


Fig. 1.—Showing glass slide in tube.

Within two to three days growth will take place on the higher side where the suspected material was planted, thus enabling a further study of the organisms or colonies present. The writer has had excellent results in using this method for the cultivation of the *bacillus bifidus*, Tissier, and the *bacillus abortus*, Bang.

To aid in this practice it is suggested that test tubes with the glass slide be made all in one piece, thus precluding all possibility of the *bacillus subtilis* contaminating the desired growths. So constructed, the tubes may be slanted in the usual way, there being no need for having one side of the slant higher than the other.